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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

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CONCEPTS OF DEPENDENCE AND ESSENTIAL DERIVATION
THE POSSIBLE USE OF DNA MARKERS

Document prepared by experts from the International Seed Federation

Slide 1

CONCEPTS OF DEPENDENCE AND ESSENTIAL DERIVATION

The possible use of DNA markers

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Madrid, September 2008

Slide 2

Outline

1. Definition of Essentially Derived Varieties in the UPOV Convention
2. ISF consideration on essential derivation
3. ISF interpretation of article 14.5 of the 1991 Act of the UPOV Convention
4. Assessment of essential derivation
5. Burden of Proof
6. Use of molecular markers, a crop-by-crop approach:
    i. Lettuce
    ii. Oilseed Rape
    iii. Ryegrass
    iv. Cotton
    v. Tomato
    vi. Maize

Slide 3

1. Definition of Essentially Derived Varieties in the UPOV Convention

"A variety shall be deemed to be essentially derived from another variety (the initial variety) when

i. It is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety;

ii. it is clearly distinguishable from the initial variety and

iii. except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

Essentially derived varieties may be obtained for example by selection of natural or induced mutants or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing or transformation by genetic engineering."

Slide 4

2. ISF consideration on essential derivation

- ISF strongly supports the concept of essential derivation
- Only few internationally agreed-upon professional rules
- Essential derivation is not a new right, but is in the scope of the right of a protected initial variety

Slide 5

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Slide 6

The technical aspect

- Clear distinctness in the sense of the UPOV Convention
- Conformity to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety
- Predominant derivation from an initial variety

  => If one of these requirements is not fulfilled, there is no essential derivation

Slide 7

The legal aspect: dependency

- The initial variety must be a protected one
- Dependence can only exist from one protected variety alone
- It is possible to have a “cascade” of essential derivation. However, a cascade of dependence does not exist

Slide 8

4. Assessment of essential derivation

Takes place after establishing that the variety is distinct (DUS) and should consider the following requirements:

- Conformity to the initial variety in the expression of the essential characteristics that result from the genotype or the combination of genotypes of the initial variety
- Predominant derivation from the initial variety

Slide 9

Proof of predominant derivation

Various criteria or combination thereof:

- Combining ability
- Phenotypic characteristics
- **Molecular characteristics**
- Breeding records

Slide 10

5. Burden of proof

For « prima facie » proof, the following elements should be sufficient:

- Strong phenotypic similarity
- Only small differences in some simply inherited characteristics
- **Strong genetic similarity**

If the owner of the i.v. has fulfilled one of the above requirements, then the second breeder would have to prove that there is no predominant derivation, or that he had not used the i.v., or a variety essentially derived from that i.v.

Slide 11

- Distance Coefficients to define a threshold (trigger point for the reversal of the burden of proof) => another interesting approach.
- ISF has mainly worked on thresholds (distances measured by molecular markers)
- Geneticists and statisticians: technically equally possible to measure distance coefficients using morphological markers; but that these distances are not always reflective of genetic distances or of pedigree relationships.
- Use of morphological characteristics could be more difficult due to environmental factors, and much more expensive.

Slide 12

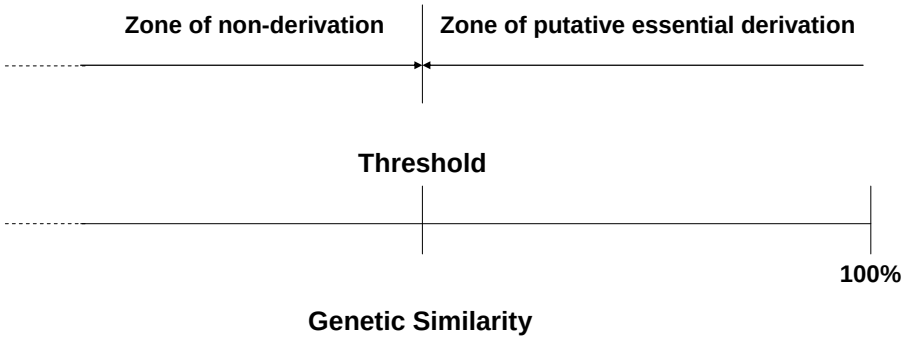
Threshold: divide the scale of conformity into two parts:

- below the threshold: no presumption of essential derivation,
- above the threshold: presumption of essential derivation and the burden of proof of non predominant derivation would fall on the breeder of the putative e.d.v..

Threshold will vary from species to species, depending on the existing genetic variability within the species and the established breeding procedures.

Slide 13

Possible use of a threshold



Slide 14

ISF recommends to its members

In any case of dispute:

- First enter into a conciliation or mediation procedure
- If that does not provide satisfactory results, enter into (binding) arbitration
- According to ISF Conciliation and Arbitration Procedure Rules .

Slide 15

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Slide 16

Choice of markers

- The markers must comply with several requirements:
  - Be “freely” available
  - Meet several technical criteria that are addressed in an ISF document “Issues to be addressed by technical experts to define molecular marker sets for establishing thresholds for ISF edv arbitration” (www.worldseed.org)

Slide 17

How to fix the threshold

- Use of pairs with known genealogy
- Similarity exceeding a percentile point in the distribution of similarities (upper-tail approach)

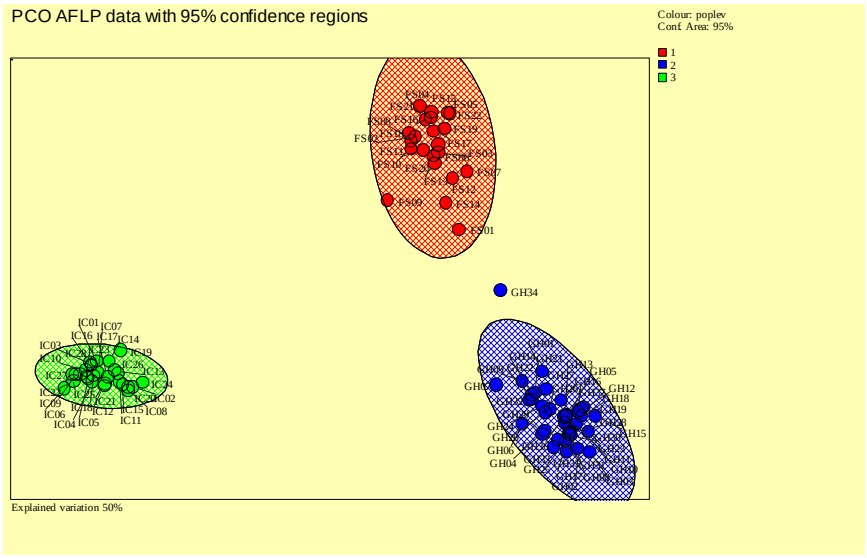
Slide 18

Lettuce EDV study

- Three lettuce types: 35 GH, 21 FS & 27 IC
- Longlist made to include most important varieties and maximum variation in each type.
- Varieties collected and shortlist made by ISF secretariat.
- In study both varieties and companies are coded.

Abbreviations:
GH: Greenhouse Heated
FS: Field Summer
IC: IceBerg

Slide 19



Madrid, September 2008 M. Bruins 19

Slide 20

zone	q	genox	genoy	compx	compy	all	errormargin
2	0.9990	22	21	5	5	0.9985	0.0028
2	0.9973	19	15	4	4	0.9878	0.0079
2	0.9956	11	8	3	3	0.9850	0.0073
2	0.9939	13	11	3	3	0.9816	0.0093
2	0.9922	10	8	3	3	0.9759	0.0095
2	0.9906	35	30	6	6	0.9740	0.0159
2	0.9889	13	8	3	3	0.9717	0.0135
2	0.9872	27	25	5	5	0.9702	0.0132
2	0.9855	12	8	3	3	0.9686	0.0154
2	0.9838	11	10	3	3	0.9684	0.0120
2	0.9822	18	16	4	4	0.9671	0.0128
2	0.9805	35	31	6	6	0.9669	0.0109
2	0.9788	12	2	3	1	0.9667	0.0141
2	0.9771	31	30	6	6	0.9651	0.0107
2	0.9754	30	18	6	4	0.9651	0.0138
2	0.9738	13	12	3	3	0.9650	0.0158
2	0.9721	12	10	3	3	0.9639	0.0142
2	0.9704	20	16	4	4	0.9619	0.0108
2	0.9687	16	14	4	4	0.9618	0.0124
2	0.9670	12	11	3	3	0.9612	0.0129
2	0.9654	35	18	6	4	0.9593	0.0169
2	0.9637	8	2	3	1	0.9587	0.0160
2	0.9620	13	10	3	3	0.9582	0.0160
2	0.9603	33	18	6	4	0.9580	0.0196
2	0.9586	35	33	6	6	0.9579	0.0154
2	0.9570	30	14	6	4	0.9570	0.0159
2	0.9553	31	16	6	4	0.9565	0.0099
2	0.9536	27	24	5	5	0.9553	0.0154
2	0.9519	20	14	4	4	0.9538	0.0165
2	0.9502	17	15	4	4	0.9536	0.0185
1	0.9486	10	2	3	1	0.9534	0.0208
1	0.9469	33	31	6	6	0.9524	0.0201
1	0.9452	33	30	6	6	0.9519	0.0152
1	0.9435	31	29	6	6	0.9511	0.0208
1	0.9418	12	3	3	1	0.9507	0.0181
1	0.9402	35	16	6	4	0.9503	0.0146

GH21 and GH22 were selected in the same F4

GH8 and GH11 come from same F3

GH30 and GH35 come from same F3

GH27 is from a cross involving GH25

Abbreviations:

q: level of conformity

genox: genotype x

genoy: genotype y

compx: company x

compy: company y

GH, Jaccard

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Slide 21

Lettuce EDV study

- 2004: 0.96 Jaccard similarity for all 3 cultigroups
- Trigger to initiate discussions => amicable settlement => arbitration => court
- Review in 5 years

Slide 22

Oilseed Rape Study

- 4 Studies carried out between 2001-2006
- Bulks of 40 plants have a very high repeatability
- Bulks of 40 plants lead to a clear separation of all the varieties
- 2007: Dice dist. of 0.85 is trigger to start discussions (assessment according to protocol)
- Review in 5 yrs

Slide 23

Ryegrass Study

- 2002 Code of Conduct adopted
- 60 plants/variety, 5 primer comb.
- Squared Euclidean distance lower than 7=> ask for arbitration
- Apply only to varieties released after adoption
- 2004 concerns by members
- New study initiated

Slide 24

Ryegrass Study

- New study SSR's (instead of AFLP's)
- Guidelines (instead of CoC)
- Apply to all varieties
- Court possible, not only arbitration
- 1st Phase: Bulks provide same result as ind. Plants
- 2nd Phase: analyse variability in current varieties => come to threshold.
- Results expected in coming months

Slide 25

Cotton Study

- Literature review on mol. mrkrs in cotton
- Gen. Div. within allotetraploid cotton varieties => no EDV threshold assigned
- Different approach: parentage
- 2007: If phen. or gen. char's suggest that 2 or more BC's were used or coefficient of parentage value is >87.5% => put. EDV
- Threshold is trigger point for discussions
- No settlement => arbitration

Slide 26

Tomato Study

- Started 2006, Daniella type
- 21 hybrids & 35 parent lines
- 93 SSR markers used for data analysis
- Dice coefficient of 0.78 between F1 and parent line : trigger point for suspected use of proprietary line in production of a hybrid. [Simple matching better?]
- Continuation with cherry type
- Results by the end of 2008

Slide 27

Maize Study

- 150 SSR markers, highly polymorphic
- Uniformly distributed, 80% coverage
- Avg 2 mrkrs/bin, Distance > 5cM
- Min. 3 alleles/mrkr, PIC min 0.3 [0.6-07.]

- 2008: At 82% conformity: burden of proof shifts to breeder of put. EDV
- At 90% conformity: strong indication of predominant derivation

Slide 28

Thank you for your attention

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ISF
International Seed Federation

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